UtahState University
CENTER FOR INTEGRATED BIOSYSTEMS
Understanding the molecules of life

DNA Sequencing

Preparation for Standard Reactions

Provide primer and template at the appropriate concentration as outlined below. This allows us to repeat a sample in case of technical failure (includ using 2ul for DNA measurement in the fluorometer). Also, evaporation by delivery should be considerable. Excess template is returned to the user upon request.

We provide universal primer:

1. Forward pUC/M13 primer:
   5' TGT AAA ACG ACG GCC AGT 3'
2. Reverse pUC/M13 primer:
   5' CAG GAA ACA GCT ATG ACC 3'
3. T7 promoter primer:
   5' TAA TAC GAC TCA CTA TAG GG 3'
4. T3 primer:
   5' ATT AAC CCT CAC TAA AGG GA 3'
5. SP6 primer:
   5' ATT TAG GTG ACA CTA TAG 3'

User should offer customer primer and templates. It is helpful if you submit templates and primers in water in the concentrations listed below for per reaction. If possible, quantitate the amount of purified DNA by measuring the absorbance at 260 nm or by some other method.

The table below shows the amount of template to use in a cycle sequencing reaction.

Template	Quantity
PCR product: 100-200 bp 200-500 bp 500-1000 bp 1000-2000 bp > 2000 bp	 1 - 3 ng 3 - 10 ng 5 - 20 ng 10 - 40 ng 20 - 50 ng
Single-stranded	25 - 50 ng
Double-stranded	150 - 300 ng
Cosmid, BAC	0.5 - 1.0 µl
Bacterial genomics DNA	2 - 3 µl

Note: 1.5 ul primer at 2 uM for each reaction.

For more information, please call us at 435-760-3688 or visit our web site at
http://www.biosystems.usu.edu/core_labs/genomics/